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Research article

# Fluorescent redox-dependent labeling of lipid droplets in cultured cells by reduced phenazine methosulfate

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ABSTRACT

Natural and synthetic phenazines are widely used in biomedical sciences. In dehydrogenase histochemistry, phenazine methosulfate (PMS) is applied as a redox reagent for coupling reduced coenzymes to the reduction of tetrazolium salts into colored formazans. PMS is also currently used for cytotoxicity and viability assays of cell cultures using sulfonated tetrazoliums. Under UV (340 nm) excitation, aqueous solutions of the cationic PMS show green fluorescence (λem: 526 nm), whereas the reduced hydrophobic derivative (methyl-phenazine, MPH) shows blue fluorescence (λem: 465 nm). Under UV (365 nm) excitation, cultured cells (LM2, IGROV-1, BGC-1, and 3T3-L1 adipocytes) treated with PMS (5 μg/mL, 30 min) showed cytoplasmic granules with bright blue fluorescence, which correspond to lipid droplets labeled by the lipophilic methyl-phenazine. After formaldehyde fixation blue-fluorescing droplets could be stained with oil red O. Interestingly, PMS-treated 3T3-L1 adipocytes observed under UV excitation 24 h after labeling showed large lipid droplets with a weak green emission within a diffuse pale blue-fluorescing cytoplasm, whereas a strong green emission was observed in small lipid droplets. This fluorescence change from blue to green indicates that reoxidation of methyl-phenazine to PMS can occur. Regarding cell uptake and labeling mechanisms, QSAR models predict that the hydrophilic PMS is not significantly membrane-permeant, so most PMS reduction is expected to be extracellular and associated with a plasma membrane NAD(P)H reductase. Once formed, the lipophilic and blue-fluorescing methyl-phenazine enters live cells and mainly accumulates in lipid droplets. Overall, the results reported here indicate that PMS is an excellent fluorescent probe to investigate labeling and redox dynamics of lipid droplets in cultured cells.

## 1. Introduction

Phenazine methosulfate (PMS) was first synthesized by Kehrmann and Havas (1913) [1] from phenazine and dimethylsulfate. Later it was applied in dehydrogenase histochemistry [2, 3, 4, 5], and studies on the photosynthesis of bacteria [6].

The hydrophilic cation PMS is now widely used as an intermediate redox reagent for coupling reduced dehydrogenase coenzymes to the reduction of tetrazolium salts into colored formazans [7, 8, 9, 10, 11, 12, 13, 14]. A similar electron-transfer agent, 1-methoxy-PMS, has also been used for revealing dehydrogenase activity in living hepatocytes [15]. Other similar but seldom scarcely used redox intermediates are Meldola blue, menadione, and pyocyanin.

In addition, PMS is also applied in cytotoxicity and viability assays of cell cultures, using sulfonated tetrazoliums such as MTS, WST-1, WST-8, and XTT [16, 17, 18, 19, 20]. PMS also accelerates the reduction of MTT

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